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12-18-00  
Date

Karen LePari  
Karen LePari

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In the application of:

Chaitan KHOSLA, et al.

Serial No.: Divisional of 08/846,247

Filing Date: Even date Herewith

For: COMBINATORIAL POLYKETIDE  
LIBRARIES PRODUCED USING A  
MODULAR PKS GENE CLUSTER AS  
SCAFFOLD

Examiner: Not Assigned

Group Art Unit: Not Assigned

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

Please change the title to read:

--METHOD TO PREPARE MACROLIDE ANALOGS--

Please amend the specification as follows:

On page 1 of the specification, line 5, please insert after "this application is" the phrase --  
a divisional of U.S. Serial No. 08/846,247 filed 30 April 1997, which is--; and on line 7, after  
"1994" please insert --which is a continuation in part of U.S. Serial No. 08/164,301, filed  
December 8, 1993, which is a continuation in part of U.S. Serial No. 08/123,732, filed  
September 20, 1993.

Please amend the claims as follows:

Please cancel claims 8-30.

Please add the following claims:

31. (Amended) A method for directing the biosynthesis of specific macrolide polyketide analogs by genetic manipulation of a polyketide-producing microorganism, said method comprising the steps of:

- (1) isolating a macrolide polyketide biosynthetic gene-containing DNA sequence;
- (2) identifying enzymatic activities associated within said gene-containing DNA sequence;
- (3) introducing one or more specified changes into said gene-containing DNA sequence which codes for one of said enzymatic activities resulting in an altered DNA sequence;
- (4) introducing said altered DNA sequence into a polyketide-producing microorganism to replace the original sequence;
- (5) growing a culture of the altered microorganism under conditions suitable for the formation of the specific macrolide polyketide analog; and
- (6) isolating said specific macrolide polyketide analog from the culture.

32. The method of claim 31 wherein said macrolide polyketide biosynthetic gene-containing DNA sequence is derived from the polyketide synthase for the production of 6-deoxyerythronolide B (6 deB).

33. The method of claim 31 wherein the altered nucleotide sequence is derived from the rapamycin PKS.

34. A method for directing the biosynthesis of a specific macrolide polyketide analog which method comprises the steps:

- (1) providing a nucleotide sequence encoding a macrolide polyketide synthase (PKS);
- (2) identifying at least one region of said nucleotide sequence that encodes an enzymatic activity;
- (3) introducing one or more specified changes into said region resulting in an altered nucleotide sequence;
- (4) introducing said altered nucleotide sequence into a microorganism;
- (5) growing a culture of said microorganism under conditions suitable for the formation of the specific macrolide polyketide analog; and
- (6) optionally isolating the specific macrolide polyketide analog from culture.

35. The method of claim 34 wherein said nucleotide sequence of step 1 encodes at least two modules of the erythromycin PKS.

36. The method of claim 34 wherein said nucleotide sequence of step 1 encodes a complete macrolide PKS.

37. The method of claim 36 wherein said nucleotide sequence of step 1 encodes a complete erythromycin PKS.

38. The method of claim 34 wherein said introducing of step 3 comprises deleting said at least one region.

39. The method of claim 34 wherein said introducing of step 3 comprises replacing said at least one region with a corresponding region of a nucleotide sequence encoding an enzymatic activity of a different macrolide PKS.

40. The method of claim 39 wherein said region encodes an enzymatic activity selected from the group consisting of ketosynthase (KS) activity; acyl transferase (AT) activity; ketoreductase (KR) activity; dehydratase (DH) activity; and enoyl reductase (ER) activity.

41. The method of claim 34 wherein said introducing of step 3 comprises mutating said at least one region.

42. The method of claim 34 wherein the macrolide PKS of step 1 is selected from the group consisting of rapamycin, avermectin, FK-506, FR-008, monensin, rifamycin, soraphen-A, spinocyn, squalestatin, and tylosin.

43. The method of claim 39 wherein the corresponding region is derived from a PKS selected from the group consisting of rapamycin, avermectin, FK-506, FR-008, monensin, rifamycin, soraphen-A, spinocyn, squalestatin, and tylosin.

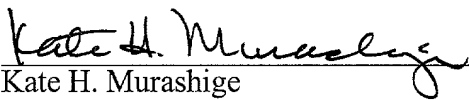
#### REMARKS

Claims 1-7 were subject to a restriction requirement in the parent application herein and represent a non-elected invention. New claims 31-43 represent more detailed descriptions of the invention of claims 1-7. Support for these claims is found, for example, on page 6 of the specification line 16 - page 7, line 18; support for claims 42 and 43 is found on page 5, line 28 - page 6, line 2; support for specific embodiments of the invention claimed is found in example 1-5. No new matter has been added and entry of the amendment is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 300622000510. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: December 18, 2000

By:   
Kate H. Murashige  
Registration No. 29,959

Morrison & Foerster LLP  
3811 Valley Centre Drive, Suite 500  
San Diego, California 92130-2332  
Telephone: (858) 720-5112  
Facsimile: (858) 720-5125